demonstrated CBMC can be ex-vivo expanded by K562 cells expressing membrane bound IL15 and 4-1BB ligand (K562-mbIL15-41BBL) resulting in specific expansion of CB NK cells and decrease in CB T-Cells.

113

A "NO-WASH" ALBUMIN-DEXTRAN DILUTION STRATEGY FOR CORD BLOOD (CB) THAW IS ASSOCIATED WITH A HIGH RATE OF ENGRAFTMENT AND A LOW INCIDENCE OF SERIOUS INFUSION REACTIONS

Rice, R.D., Abboud, M., Hawke, R.M., Schaible, A.M., Heller, G., Scaradavou, A., Barker, J.N. Memorial Sloan-Kettering Cancer Center, New York, NY

The albumin-dextran dilution with centrifugation ("wash") for CB thaw used for children has been adopted for adults as a matter of convention. An alternative CB dilution without centrifugation may be advantageous for adults in whom any cell loss may be significant. In contrast to a bedside thaw, it is still conducted in the controlled laboratory environment. Therefore, we conducted a prospective study of CB transplantation (CBT) using albumin-dextran dilution with the hypothesis that the "no wash" thaw would be associated with tolerable infusion reactions and a high rate of engraftment. Recipients ≥20 kg were eligible. Patients [n = 50; median 42 years (range 7–66); median 72 kg (range 24–109)] were transplanted for high-risk hematological malignancies with ablative (n = 34) or non-ablative (n = 16) conditioning and double unit grafts to augment engraftment. 99 units were thawed with albumin-dextran dilution (1 RBC replete unit was washed). Units were 6/6 (n = 5), 5/6 (n = 52) and 4/6 (n = 42) HLA-A,B antigen, DRB1 allele matched to the recipient. A 5:1 Gentran®40/ 25% albumin solution was used. RBC deplete units (n = 96) were diluted ≥5.5 fold [median 200mls (range 200–500)]. The larger volume RBC replete units (n = 3) were diluted ≥4 fold [median 400mls (range 400–535)] using double tubing to connect to the transfer pack to prevent clogging. All patients received pre-medication and pre- and post-hydration. There were no serious adverse events related to infusion. 18 had no reactions and 32 had reactions requiring additional therapy (2 transient hypoxia, 29 hypertension, 6 nausea, 3 pain, 1 rigor/fever). Also, 1 had transient bradycardia and 6 had transient creatinine elevation. Cumulative incidence (CI) of sustained donor engraftment was 96% (95%CI:90–100) with neutrophil recovery at a median of 25 days (range 13–43) in ablative and 11 days (range 7–36) in non-ablative recipients. CI of day 100 grade II-IV acute GVHD was 40% (95%CI:26–54), and 6 patients have chronic GVHD to date. CI of day 180 transplant-related mortality is 24% (95%CI:11–37) and 1 year overall survival is 63%(95%CI:49–78). In conclusion, the dilution thaw technique is an alternative to wash in patients ≥20 kg with cell dose yields and clinical outcomes that compare favorably to a traditional wash. This technique is more efficient, reduces unit manipulation, speeds time to infusion, reduces cell loss, and may be the thaw technique of choice for adult CBT.

Post-Thaw Cell Dose Yields

	Pre-thaw TNC x 10^7/kg	Post-thaw TNC x 10^7/kg	% TNC Recovery	CD34+ Viability	Post-thaw CD34+ x 10^5/kg/unit	Post-thaw CFU-GM x 10^4/kg/unit
Median	2.65	2.25	86%	90%	0.90	1.25
Range	1.06–6.87	0.92–5.36	50–121%	34–98%	0.13–3.69	0.00–4.38

114

COLONY-FORMING-UNIT (CFU) ASSAY WITH HIGH-RESOLUTION DIGITAL IMAGING: A RELIABLE SYSTEM FOR CORD BLOOD (CB) CFU EVALUATION

Albano, M.S., Stevens, C.E., Dobrila, L.N., Scaradavou, A., Rubinstein, P. New York Blood Center, New York, NY

Among CB graft characteristics currently used as determinants of quality and engraftment potential, only the CFU assay evaluates the functional state and number of hematopoietic progenitor cells. Traditionally, classification and enumeration of CFU colonies are performed manually by light microscopy, and the assay is time consuming, subjective and difficult to standardize. We developed a strategy combining high resolution digital imaging and colony staining with MTT, which allows testing of large numbers of CB samples in a "high throughput" approach. CFU growth was assessed in 2159 CB units; samples were obtained after processing (AXP® system). Our new approach permits a more precise analysis, classification, and enumeration of the different colonies, and standardization of the assay. Furthermore, stored CFU images can be reviewed prior to the release of a CBU for transplantation. The number of CFUs (179 ±108/uL) correlated strongly with Total Nucleated Cell count (R^2: 0.33), Mononuclear Cell count (MNC +nucleated RBC, R^2: 0.35), and especially with pre-processing CD34+ cell content (R^2: 0.8, all $p<0.001$). Comparison with results obtained by the traditional CFU assay when testing was performed on pre-processing samples showed minimal difference (10–15%) in the CFU/uL or CFU/MNC ratio. CFU evaluation from a frozen-thawed segment attached to the CBU bag is now underway. Even though a small number of segments were tested so far (n = 15), CFU/uL correlated with the CBU's respective pre-freeze CD34+ cells (R^2: 0.78), and with the post-thaw CD34+ cells from the same segment (R^2: 0.74). CD34+ cell viability was >93% in all segments. In summary, CFUs can be enumerated reliably from CB samples with a new strategy that can be standardized. The feasibility of CFU growth from thawed segments was also evaluated, and CFU yield correlated strongly with post-processing and segment-derived CD34+ cell counts. This assay can be used as a practical quality control measure of the cryopreservation process prior to a CBU release for transplantation. Studies are in progress to assess whether CFU yield from a segment could predict engraftment and, therefore, used for the selection of CB units for transplantation.

115

REGULATORY T CELL S (Tregs) CAN BE ISOLATED FROM G-CSF MOBILIZED PBSC AFTER MONOCYTE DEPLETION AND INHIBIT ANTI-STEM CELL T CELL ALLOREACTIVITY

Mahmud, D., Rondelli, D. University of Illinois at Chicago, Chicago, IL

Small numbers of human CD4+CD25+FoxP3+ Tregs can be isolated from normal peripheral blood, thus their potential clinical application is limited. In this study we tested whether Granulocyte Colony-Stimulating Factor (G-CSF)-mobilized peripheral blood stem cells (PBSC) from healthy donors can represent a useful source of CD4+CD25+ cells with regulatory activity. We utilized antibodies conjugated to microbeads (Miltenyi Biotec Inc, Auburn, CA) to immunomagnetically separate the cells on a MidiMACS device (Miltenyi) and checked the purity after isolation by flow cytometry. Starting from on average $4.0 \pm 1.8 \times 10^8$ unseparated PBSC (n = 3) we positively selected $2.4 \pm 0.8 \times 10^6$ CD34+ cells (>90% purity). We then utilized the CD34- cell fraction to isolate Tregs. Due to the large content of CD4dim monocytes in the initial cell product, we initially depleted the PBSC of CD14+ cells and then utilized a two-step process that includes a CD4+ cell negative selection (using a cocktail of biotin-conjugated antibodies against CD8, CD14, CD19, CD16, CD36, CD56, CD123, TCR γ/δ and Glycophorin-A) followed by a positive selection of CD25+ cells (Treg isolation kit, Miltenyi). This process allowed us to obtain $1.2 \pm 1 \times 10^6$ CD4+CD25+ cells with a purity of >70%. Intracellular expression of FoxP3 was also detected in purified CD4+CD25+ cells by flow cytometry. Primary mixed leukocyte cultures (MLC) were performed with irradiated CD34+ cells isolated from PBSC and HLA mismatched blood CD3+ responders for 6 days and T cell response was measured by a 3H-thymidine uptake assay. Tregs isolated from PBSC were added to the MLC at 1:2 Treg:responder ratio to test their regulatory function. Control experiments were performed using CD4+CD25- cells. Addition of Tregs isolated from PBSC resulted in 76 ± 17% inhibition of anti-CD34 T cell alloreactivity (cpm: 19000 ± 530 vs 4590 ± 1880) (n = 3), while control CD4+CD25 neg cells did not show suppressive activity. These findings show that after isolation of CD34+ cells, adequate numbers of Tregs can be obtained from the CD34- cell fraction of PBSC by using a three-step process. In addition, since Tregs isolated from PBSC suppressed in-vitro T cell alloreactivity against CD34+ cells, these findings will prompt the design of pre-clinical studies to test the combination of PBSC-derived CD34+ cells and Tregs in HLA mismatched transplantation.